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PATENT

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Application Number 10/686,548

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Bauer et al.
Application No. 10/686,548
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Confirmation No.
For: POSITIVE DETECTION LATERAL-FLOW APPARATUS AND METHOD FOR SMALL AND LARGE ANALYTES
Examiner:
Art Unit:
Attorney Reference No. 6122-66637-01

CERTIFICATE OF MAILING

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Attorney or Agent for Applicant(s) William D Moore

Date Mailed May 17, 2005

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DECLARATION OF ROBERT L. BUCK UNDER RULE 132

1. I hold a Ph.D. in Biochemistry and have worked for 22 years in the field of medical diagnostics. I am currently the Director of Research and Development at a biotechnology company in Portland, Oregon.

2. I have performed experiments on behalf of A-Fem Medical Corporation concerning a test strip that is described and claimed in U.S. Patent Application No. 10/686,548. This Declaration under Rule 132 ("Declaration") describes those experiments and the results that were obtained. These experiments demonstrate that the initial binding of analyte to the antibody in the primary capture zone occupies the antibody binding sites, such that subsequently arriving conjugate (analyte analog-linker-tracer or ALT) will more reliably migrate through the primary capture zone to the secondary zone.

3. In summary, I used nitrocellulose lateral flow strips in which a path of liquid flow was defined along a bibulous substrate from a sample application pad to a mobilization zone to a primary capture zone and then to a secondary capture zone. The primary capture zone contained anti-morphine antibody and the secondary capture zone contained streptavidin. The fluorescent latex particles were placed 20 mm, 13 mm and 4 mm upstream from the primary capture zone. A 2000 ng/ml morphine urine sample was applied to the sample pad, and the fluorescent particles were clearly seen moving behind the solvent front when the fluorescent particles were positioned at 20 mm and 13 mm from the primary capture zone, but not when they were only 4 mm upstream. Since the fluorescent latex particles were coated only with morphine conjugate and not biotin, only the primary capture zone intensity was viewed and measured. The intensity of the fluorescent signal from the primary capture zone was used as an indication of the sensitivity of the test. The weaker the signal from the primary capture zone, the greater the signal would be expected to be from the secondary capture zone (because the tracer would pass

through the primary zone where the fixed number of antibody binding sites had already bound the analyte). We found that a greater distance between the primary capture zone and the position at which the latex particles was applied resulted in a lower signal emanating from the primary capture zone, when the signal was either determined visually or instrumentally. Since differential migration increases as the distance to the primary capture zone increases, this indicates that differential migration increases sensitivity of the test.

4. The full experimental protocol for these experiments was as follows:

Fluorescent latex particles (2.5 µl of 10 mg/ml solution in conjugate diluent) were added to 3 positions on the strip, at varying distances from the primary capture zone. The first zone was centered 20 mm from the primary capture zone, and was positioned on the top edge of the sample pad. The second zone was centered 13 mm from the primary capture zone, and was positioned in the middle of the conjugate pad. The third zone was centered 4 mm from the primary capture zone, between the primary capture zone and the beginning of the nitrocellulose section of the strip.

Latex was not allowed to dry. To the front of the sample pad was placed in 25 µl increments a total of 100 µl of either negative or 2000 ng/ml urine control (Quick-Check) to avoid flooding. Strips were allowed to develop, and the intensity of the primary capture zone was determined visually using a Model UVGL-58 Minerlight Lamp, 254/366 nm, (UVP Inc., Upland, CA) at the 366 nm setting. Scoring was on a scale from 0-3, where a value of 3 was the most intense band seen, and zero indicated no visible fluorescence was seen. In addition, the primary capture zone bands were cut from 2000 ng/ml morphine strips, and fluorescence intensity measured in the Abbott TDx (Photo Cal mode), using 1.0 ml of 0.10% Tween-20 in 10 mM Tris, containing 0.2% TEA to dissociate antibody-latex complexes. Three control strips were cut between the primary capture zone and secondary capture zone, and subtracted as background.

Visual Results of Fluorescent Latex Experiment				
2000 ng/ml morphine urine control				
		20 mm	13 mm	4 mm
Strip 1		0.5	1	3
Strip 2		0	1	3
Strip 3		0.5	1	3
Negative morphine urine control				
		20 mm	13 mm	4 mm
Strip 1		3	3	3
Strip 2		3	3	3

TDx Results of Fluorescent Latex Experiment			
2000 ng/ml morphine urine control			
	20 mm	13mm	4 mm
Average (gain 20 less background)	96	228	392

5. In addition, I have observed that covalent coupling of a tracer (such as a colored latex bead) to an analyte or analyte analog in the claimed assay produces a more reliable assay. Passively coating a tracer to a latex bead more readily allows the tracer to separate from the analyte or analog, such that the analyte or analog can function as "free analyte" and provide falsely elevated values in a positive reporting system.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

By Robert L. Buck
Robert L. Buck, Ph.D.

Date 15 May 05